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Retinal Removal Up-Regulates Cannabinoid CB_1 Receptors in the Chick Optic Tectum

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The endocannabinoid system has been implicated in several neurobiological processes, including neurodegeneration and neuroprotection. The aim of this study was to evaluate the effects of unilateral retinal ablation on the expression of the cannabinoid receptor subtype 1 (CB_1) at both protein and mRNA levels in the optic tectum of the adult chick brain. After different survival times postlesion (2–30 days), the chick brains were subjected to immunohistochemical, immunoblotting, and real-time PCR procedures to evaluate CB_1 expression. TUNEL and Fluoro-Jade B were used to verify the possible occurrence of cell death, and immunostaining for the microtubule-associated protein MAP-2 was performed to verify possible dendritic remodeling after lesions. No cell death could be observed in the deafferented tectum, at least up to 30 days postlesion, although Fluoro-Jade B could reveal degenerating axons and terminals. Retinal ablation seems to generate an increase of CB_1 protein in the optic tectum and other retinorecipient visual areas, which paralleled an increase in MAP-2 staining. On the other hand, CB_1 mRNA levels were not changed after retinal ablation. Our results reveal that CB_1 expression in visual structures of the adult chick brain may be negatively regulated by the retinal innervation. The increase of CB_1 receptor expression observed after retinal removal indicates that these receptors are not presynaptic in retinal axons projecting to the tectum and suggests a role of the cannabinoid system in plasticity processes ensuing after lesions.

Key words: retinal ablation; cannabinoid system; optic tectum; visual pathways

The endocannabinoid system comprises endocannabinoid molecules, their synthetic and degradation enzymes, and their receptors, and it has been implicated in several neural processes, such as cognition, antinociception, sleep, feeding, and modulation of synaptic transmission of other neurotransmitter systems (Matsuda et al., 1990; Piomelli, 2003; Di Marzo and Matias, 2005; Chevaleyre et al., 2006; Hashimotodani et al., 2007). The modulator role of cannabinoid system has been suggested in several studies aimed at determining the anatomical distribution of the receptors and analyzing the pharmacological effects of cannabinoid compounds (Pertwee, 1997, 2001; Ameri, 1999; Fernandez-Ruiz et al., 2000; Fride, 2002, 2004; Howlett et al., 2002, 2004). For instance, the predominant presynaptic localization of cannabinoid receptors suggests their participation in retrograde signaling at γ-aminobutyric acid (GABA)-ergic and glutamatergic neurons, with consequences for plasticity processes such as learning and memory (Freund et al., 2003; Piomelli, 2003). In addition, the endocannabinoid system has also been implicated in brain development, neuroprotection, and plasticity processes (Fernandéz-Ruiz et al., 2000; Grundy et al., 2001; Panikashvili et al., 2001, 2005, 2006; Parmentier-Batteur et al., 2002; Ramos et al., 2002; Mato et al., 2003; Ashton et al., 2004; Begbie et al., 2004; Karanian et al., 2005; Leonelli et al., 2005; Chevaleyre et al., 2006; Gilbert et al., 2007).

Several studies demonstrated increased levels of endocannabinoids and up-regulation of CB_1 receptors in brain damage models in rats and mice (Romero et al., 2000; Hansen et al., 2001; Panikashvili et al., 2001; van der Stelt et al., 2001; Unzicker et al., 2005). The observed accummulation of endocannabinoids and CB_1 up-regulation could represent a protective response contributing to rescue neurons from death. Taken together with the receptor reduction observed in neurodegenerative diseases such as Huntington's disease (Glass et al., 2000) and the symptomatic relief provided by cannabinoids in experimental models of chronic neurodegenera-

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tive diseases (Grundy et al., 2001), those data are suggestive of a neuroprotective effect of cannabinoids.

The endocannabinoid system has also been pointed out as an important system in plasticity processes of the central nervous system. Karanian and collaborators (2005) describe the participation of CB_1 receptor in the generation of important signals for the maintenance of synapses in the hippocampus of rats. In summary, cannabinoids activate pathways related to cell survival and inhibit signals that could compromise synaptic integrity, involving endogenous compensatory systems. Another study suggests that the CB_1 receptor could contribute to the neurochemical control of vestibular system plasticity after unilateral deafferentation, without requiring changes in the CB_1 protein expression (Ashton et al., 2004). In addition, Tagliaferro and collaborators (2006) demonstrate changes of cytoskeletal and synaptic density elements in several rat brain regions after chronic treatment with a CB_1 receptor agonist, again suggesting the involvement of the cannabinoid system in neural plasticity.

As part of a general effort to understand the central consequences of retinal lesions (Britto et al., 1994; Pires et al., 1998, 2000; Torrão and Britto, 2004), the aim of this study was to verify the effects of unilateral retinal ablation on the expression of the CB_1 receptor in primary visual areas of the chick brain, especially the optic tectum (Leonelli et al., 2005). We also tested the presumptive occurrence of cell death after retinal ablation using the in situ DNA fragmentation staining method, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-fluorescein nick end labeling (TUNEL; Gavrieli et al., 1992), and the Fluoro-Jade B technique (Schmued and Hopkins, 2000). In addition, microtubule-associated protein-2 (MAP-2) immunohistochemistry was used to verify possible dendritic changes after deafferentation. The chick visual system is especially well suited for such an analysis, insofar as there is a virtually complete crossing of retinal fibers, which generates control and experimental tecta in the same subject (Britto et al., 1994; Torrão and Britto, 2004). Furthermore, both retinal neurons and central visual structures express CB_1 receptors in several vertebrate classes (Porcella et al., 1998; Bisogno et al., 1999; Straiker et al., 1999a,b; Yazulla et al., 1999; Leonelli et al., 2005). The present approach generated a framework of data that may be suitable to test the role of endocannabinoids in plasticity processes.

MATERIALS AND METHODS

Retinal Lesions

Seventy-nine 15-day-old chicks (*Gallus gallus*) were obtained from a local hatchery and used in this study. The chicks were anesthetized with ketamine (5 mg/100 g of body weight, i.m.) and xylazine (1 mg/100 g, i.m.) and subjected to unilateral retinal ablation as described previously (Torrão and Britto, 2004). Briefly, the lens and vitreous were removed, and the neural retina was completely destroyed and removed with cotton swabs. A piece of absorbable gelatin (Gelfoam; Upjohn, Kalamazoo, MI) was placed inside the eye, and the eyelids were sutured. Most of these animals were used for CB_1 and MAP-2 immunohistochemistry (n = 24) or TUNEL and Fluoro-Jade B studies (n = 15). To obtain some additional information on CB_1 protein and mRNA expression, additional groups of chicks were used for immunoblotting (n = 28) and for RT-PCR (n = 12) experiments, respectively. The experiments were carried out in accordance with the guidelines of the Brazilian College for Animal Experimentation (COBEA) and were approved by The Ethics Committee for Animal Research of the University of São Paulo.

Perfusion

After distinct survival times postlesion (2, 7, 15, and 30 days), the chicks were deeply anesthetized with ketamine and xylazine and perfused through the heart with phosphate-buffered saline (PBS) and 2% (for immunohistochemistry) or 4% paraformaldehyde (for TUNEL and Fluoro-Jade B) in 0.1 M phosphate buffer (PB; pH 7.4). Brains were removed from the skull and postfixed for 3 hr for immunohistochemistry or 6 hr for histochemical studies, then transferred to a 30% sucrose solution in PB to ensure cryoprotection. Coronal sections (30 μm) of the frozen brains were cut on a sliding microtome and subjected to immunohistochemistry, TUNEL, and Fluoro–Jade B.

TUNEL and Apoptosis

Brain sections were mounted on gelatin- and chromoalumen-coated slides, washed in 0.05 M PBS (pH 7,4), and then incubated in a solution containing 0.1% Triton X-100 and 0.1% sodium citrate in 0.05 M PBS for 2 min in ice. The material was washed and incubated with a TUNEL kit mixture (Roche Molecular Biochemicals, Mannheim, Germany) for 60 min at 37°C. After the reaction, the sections were washed and, coverslipped using VectaShield (Vector, Burlingame, CA), and analyzed on a fluorescence microscope equipped with a standard fluorescein filter.

Fluoro-Jade B and Neurodegeneration

Brain sections were mounted on gelatin- and chromoalumen-coated slides and air dried on a slide warmer. The material was immersed in a solution containing 1% sodium hydroxide in 80% alcohol for 5 min and in 70% alcohol followed by distilled water for 2 min. The slides were then transferred to a solution of 0.06% potassium permanganate for 10 min, under agitation, to ensure reduction of background staining and washed in distilled water for 2 min. The material was then incubated in a solution of 0.001% Fluoro-Jade B (Chemicon, Temecula, CA) in 0.1% acetic acid for 20 min, and washed three times in distilled water. The slides were placed on a slide warmer until they were fully dry, cleared by immersion in xylene for 2–3 min, and coverslipped using DPX (Fluka, Milwaukee, WI). Finally, the material was analyzed on a fluorescence microscope equipped with a standard fluorescein filter.

Immunohistochemistry

Free-floating sections were incubated overnight with a rabbit polyclonal antiserum against the extracellular N-terminal portion of the CB_1 receptor (Cayman, Ann Arbor, MI) diluted 1:1,000 or a mouse monoclonal antibody against MAP-2 (Chemicon) diluted at 1:2,000. Both antibodies were diluted in PB containing 0.3% Triton X-100 plus 5% of normal goat (for the rabbit polyclonal antiserum) or normal donkey sera (for the mouse monoclonal antibody). After three washes in PB, the sections were incubated for 2 hr with a biotinylated goat anti-rabbit or donkey anti-mouse sera (Vector) diluted 1:200 in PB containing 0.3% Triton X-100. The sections were washed again in PB and incubated for 1 hr with the avidin-biotin-peroxidase complex (ABC Elite; Vector). The sections were then reacted with 0.05% 3-3′-diaminobenzidine and a 0.01% solution of hydrogen peroxide in PB and intensified with 0.05% osmium tetroxide in water. Finally, the sections were mounted on gelatin- and chromoalumen-coated slides, dehydrated, cleared, and coverslipped with Permount (Fisher, Pittsburgh, PA). Controls for the specificity of staining included the omission of the primary antibody and substitution of the primary antibody for appropriate normal sera. The material was then analyzed with a light microscope, and digital images were collected.

A densitometric analysis was performed on the immunolabeled material in NIH Image software. The average optical density of labeled areas of the control and experimental optic tecta for CB_1 receptor immunoreactivity for each time point was evaluated with the routine ''density slice''. In summary, nine tectal fields in total in three sections/animal through the superficial layers of the optic tectum were subjected to quantitative analysis. Mean values ± SEM obtained from densitometric analysis were statistically analyzed by two-way ANOVA followed by the Tukey test. For all comparisons, $P < 0.05$ was considered significant.

Immunoblotting

After the same survival times as described above, the chicks were sacrificed by cervical dislocation, and the tecta were rapidly collected and homogenized at 4°C in an extraction buffer (Tris, pH 7.4, 100 mM; EDTA 10 mM; PMSF 2 mM; aprotinin 0.01 mg/ml). The homogenates were subjected to centrifugation at 12,000*g* for 20 min, and the protein concentration of the supernatant was determined using a protein assay (Bio-Rad, Hercules, CA). Samples from the homogenate containing 75–100 μg protein were subjected to a 10% acrylamide gel containing sodium dodecyl sulfate and electrotransferred to nitrocellulose membranes using a Trans-Blot cell system (Bio-Rad). The nitrocellulose membranes were then blocked for at least 2 hr and incubated overnight with the same antibody against CB_1. Loading control with β-actin was conducted in all experiments by using an anti-β-actin antibody (Sigma, St. Louis, MO). The specifically bound antibody was visualized using a chemiluminescence kit (ECL Kit; Amersham Biosciences, Little Chalfont, Buckinghamshire, United Kingdom). Finally, the blots were densitometrically analyzed in Scion Image 4.0.2 (Scion Corporation, Frederick, MD) and statistically treated as described for immunohistochemistry. Because there were no β-actin changes after retinal ablation under our conditions, the optical density of the CB_1 bands was first normalized in relation to the corresponding β-actin bands in each experiment. Subsequently, the normalized data were treated to evaluate protein changes in the experimental tecta in relation to their controls for each time point. The data were then subjected to a two-way analysis of variance followed by the post hoc Tukey test as described above.

Real-Time PCR

After survival times of 2, 7, and 15 days, which seemed to produce noticeable effects in preliminary immunohistochemical and immunoblotting experiments, a group of animals was sacrificed for RNA extraction by cervical dislocation, and each optic tectum (total of 100 mg) was rapidly collected and total RNA isolated using the Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA), following the manufacturer's recommendations. These samples were dissolved in free ultrafiltered water, and their concentrations were determined by measuring absorbance at 260 nm. The purity of the RNA was determined by calculating the 260/280 nm ratio, and the RNA integrity was checked on a 1% agarose gel stained with ethidium bromide. Reverse transcription (RT) reaction was done with 5 μg of the total RNA containing oligo-dt (500 μg/ml), 10 mM of each dNTP, 5× First-Strand Buffer, 0.1 M dithiothreitol (DTT), and 200 U reverse transcriptase (SuperScript II; Invitrogen). RT reaction was performed at 70°C for 10 min, followed by 42°C for 60 min and 10 min at 95°C.

Oligonucleotide primers were designed in Primer Express Software (Applied Biosystems, Foster City, CA) and synthesized by Dialab (São Paulo, Brazil). The sequences used were: cannabinoid receptor type 1 gene from *Gallus gallus* (forward: 5′-TATCCTCCACTCCCGAAGCCTG-3′; reverse: 5′-AAACGCAACGACAGCCTTTGG-3′) and β-actin as an endogenous control (forward: 5′-CCAACACAGTGC TGTCTGGTGG-3′; reverse: 5′-TTTGCGGTGGACAATG-GAGG-3′).

The real time PCR amplifications were performed using 10–80 ng/μl of each RT reaction product diluted in a reaction buffer containing 25 μl of Sybr green (Invitrogen, Carlsbad, CA) as fluorescent dye, 200 nM primers (forward and reverse) in a final volume of 50 μl per sample, divided in two wells (duplicate). Cycling conditions were set as follows: an initial activation step at 95°C for 15 min, 40 cycles of 15 sec of denaturation at 95°C, and 60 sec of annealing at 58°C, then melt curve analysis was performed by heating samples from 65°C to 99°C (1°C increment changes at 5-sec intervals). The relative values of mRNA levels of the tested gene (CB_1), comparing all samples and controls in duplicate, is based on real-time detection of PCR products by measuring fluorescence quantified with the Rotor Gene 3000 equipment (Corbett Research, Mortlake, Australia), based on current methodology (Bustin, 2000). The relative quantification value of each target gene was analyzed using a comparative Ct method (Livak and Schmittgen, 2001). All quantifications were normalized to an endogenous control gene (β-actin). The Ct values of samples and controls were adjusted initially

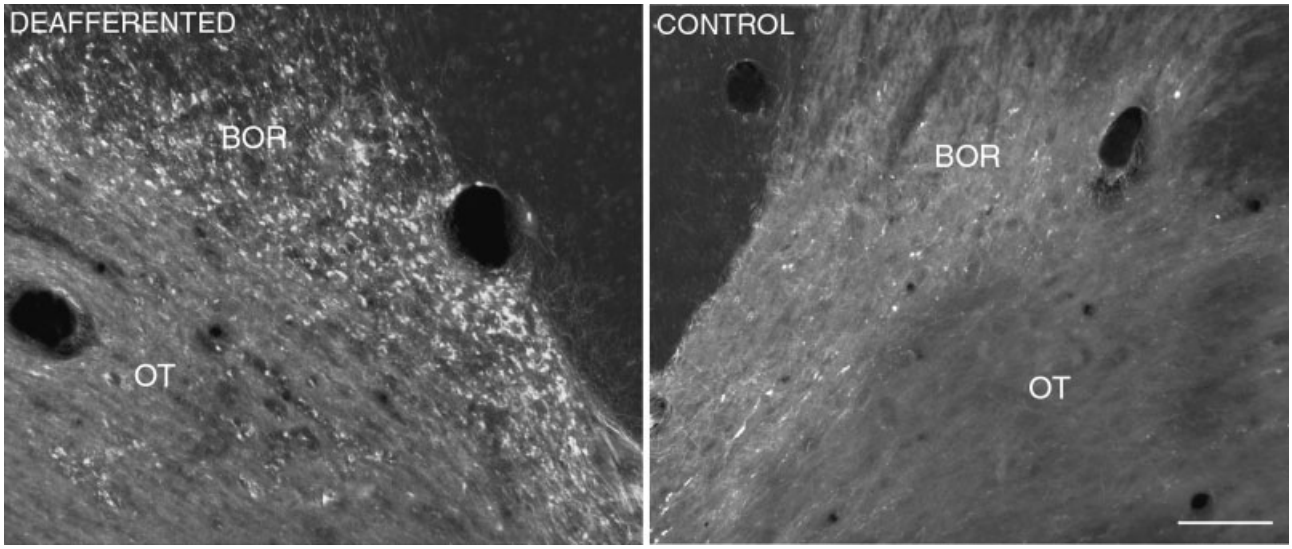

Fig. 1. Histochemistry for Fluoro-Jade B in fibers of retinal origin located in the main optic tract (OT) and the basal optic root (BOR). Staining in the deafferented side is indicative of fiber degeneration. A few fibers and varicosities are also seen in the control side and probably represent a small contingent of degenerating ipsilateral retinal fibers. Scale bar = 100 μm.

TABLE I. Effects of Retinal Ablation on CB_1 Immunoreactivity in the Chick Optic Tectum[†]

CB_1 staining (optical density)	2 Days (n = 7)	7 Days (n = 6)	15 Days (n = 6)	30 Days (n = 5)
Control	311.0 ± 10.48	230.9 ± 7.99	240.0 ± 9.66	142.5 ± 12.31
Deafferented	332.0 ± 10.71	353.2 ± 10.26★	346.2 ± 11.48★	250.6 ± 15.42★

[†]Data are given as average of the absolute numbers of the CB1 optical density and standard error of the means. The control always refers to the ipsilateral tectum in relation to the ablated retina, and deafferented to the contralateral side. The data were submitted to Paired *t*-test. The comparisons and analyses of significance involved only the control with the deafferented side of the same survival period. The apparent variation of data for the control side probably was due to the increase of the optic tectum with age. (please see text).
★$P < 0.001$.

for the amount of the CB_1 gene, β-actin gene (ΔCt; Ct of sample or control − Ct of β-actin), and then compared with the medium control by subtracting its dCt to yield a ΔΔCt. The final values for samples are reported as a -fold difference relative to the expression of the mean of the control (calculated as $2^{-\Delta\Delta Ct}$), with the mean of the control arbitrarily set to 1. Results were expressed as the ratio of the mRNA level of the CB_1 gene in relation to the mRNA level of β-actin. Mean values ± SEM were statistically analyzed using the two-way ANOVA followed by the Tukey test as described above.

RESULTS

TUNEL and Fluoro Jade B

Retinal ablation does not seem to generate cell death by apoptosis in the optic tectum; the TUNEL method used here showed no labeled nuclei in either the contralateral or the ipsilateral optic tectum (data not shown). On the other hand, a neurodegenerative process could be identified in retinal axons and their central terminations in visual areas by the Fluoro-Jade B method. This process was characterized by the presence of stained fibers and puncta and was especially noticeable after 7 days postlesion (Fig. 1).

CB_1 Protein and mRNA Expression in the Optic Tectum After Retina Removal

In general, CB_1 immunoreactivity was observed almost exclusively in the neuropil of visual structures. In the control optic tectum, the staining was seen mainly in the most superficial layers, specifically in Cajal's layers 2–3 and 5, and was more intense in layers 2–3. The deepest layers showed a moderate immunoreactivity for CB_1.

The unilateral retinal ablation produced an increase in the immunoreactivity for CB_1 in the neuropil of superficial layers of the contralateral (deafferented) optic tectum, in all layers containing CB_1. These results were observed for all survival times, and the statistical data for the mean optical density of staining are summarized in Table I.

The retinal ablation produced marked effects on the CB_1 immunostaining, namely, an increase of about 56% (Fig. 2), 52%, and 93% after 7, 15, and 30 days, respectively. However, it should be stressed that a marked reduction in the size of optic tectum generated

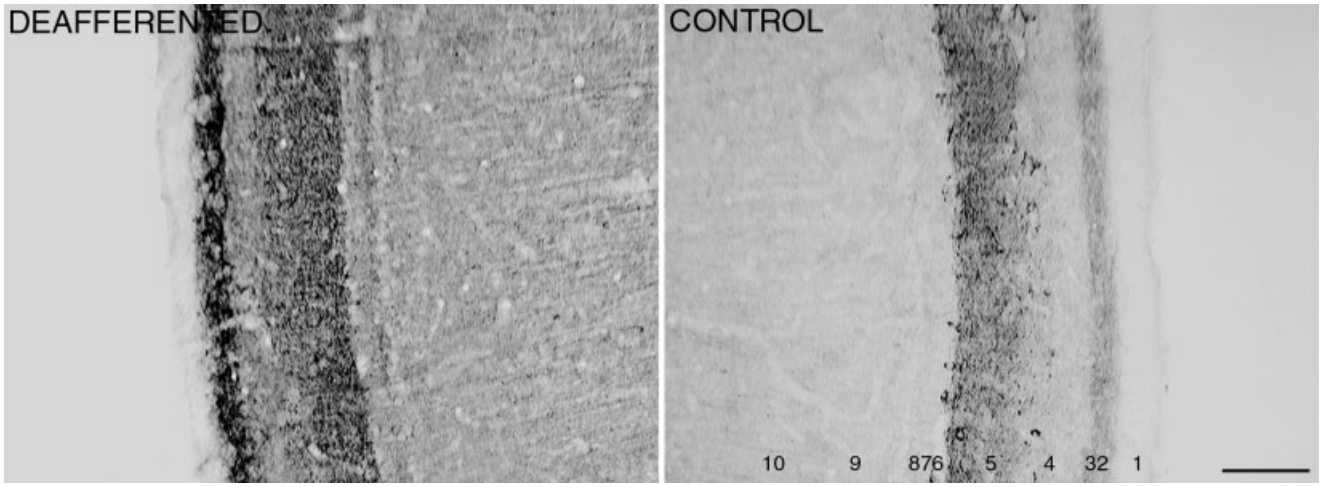

Fig. 2. Immunohistochemical staining for CB_1 in the chick optic tectum 7 days after unilateral retinal ablation. Staining in the deafferented side is markedly increased. Numbers refer to tectal layers, according to Cajal's scheme. Scale bar = 250 μm.

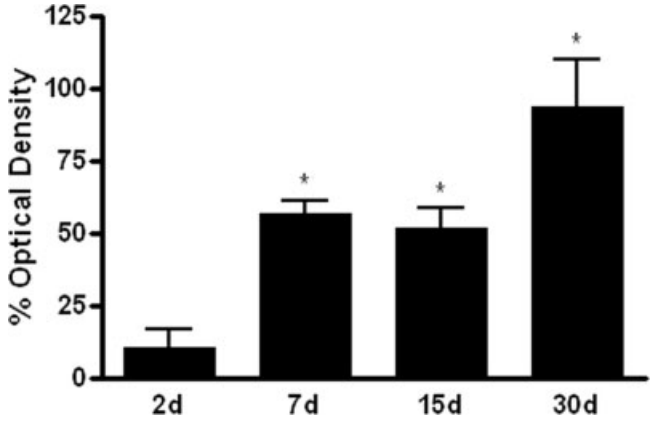

Fig. 3. Effects of unilateral retinal ablation upon the CB_1 protein expression in the chick optic tectum evaluated by immunohistochemistry. The graph depicts the percentage changes of CB_1 expression after 2, 7, 15, and 30 days postablation in relation to the control optic tecta for each time point. Expression of CB_1 exhibited a statistically significant increase after 7, 15, and 30 days ($^{\star}P < 0.001$). It should be mentioned that a marked tectal shrinkage occurred after 30 days postlesion (see text).

by the ablation after longer survival times (especially 15 and 30 days) was clearly visible, and this shrinkage has not been considered when analyzing the immunolabeling results. After 15 days postablation, the shrinkage of tectal layers in the contralateral side was about 31%, and after 30 days it was about 47%.

The optical density data actually revealed an apparent decrease of immunostaining in the control tectum along the time points, which could suggest a reduction of CB_1 with age. However, insofar as this effect was not observed for the immunoblotting data (see below), it is likely that the decrease in optical density is related to the increase in the optic tectum with age.

This increase rendered difficult the comparison of absolute optical density values along the different time points tested. The normalized values of the CB_1 increase after retinal ablation shown in Figure 3 reveal, however, that the CB_1 up-regulation was very clear along the different time points, regardless of the optic tectum size. The normalized data for the effects of unilateral retinal ablation after all survival times are depicted in Figure 3.

As observed with the immunohistochemical method, the unilateral retinal ablation also appeared to produce changes in CB_1 expression in the immunoblotting experiments (Fig. 4). The optic tectum contralateral to the retinal ablation showed a significant increase of CB_1 expression in relation to the control tectum after 7 and 15 days of about 22% (22.19% ± 8.67%, $P < 0.05$) and about 50% (50.06% ± 15.84%, $P < 0.01$), respectively. Unlike what was observed for protein levels, unilateral retinal ablation did not produce any detectable changes of CB_1 mRNA levels after 2, 7, or 15 days of survival time (Fig. 5).

MAP-2 Expression in the Optic Tectum After Retina Removal

MAP-2 expression was detected in the control tectum as stained processes encompassing several layers of the superficial and intermediate tectum, especially in layers 2–4, 5b, and 8–10. In the intermediate layers, the most prominent staining occurred in radial dendrites, which sometimes reach the superficialmost layers 2–3. All of those tectal layers included occasional labeled perikarya. After retinal lesions, a marked increase of MAP-2 staining was observed in the deafferented tectum. This increase was more pronounced in layers 2–3 and especially 5b, whereas the vertical dendrites appeared to be reduced in their numbers and partially to lose their radial

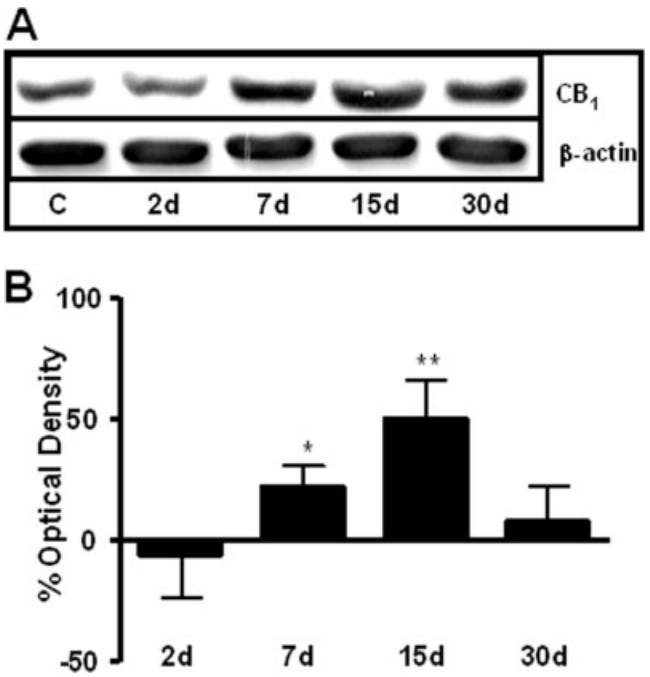

Fig. 4. Effects of unilateral retinal ablation on the CB_1 protein expression in the chick optic tectum evaluated by immunoblotting experiments. **A:** Representative immunoblots of CB_1 of the control tectum (C) and at 2, 7, 15, and 30 days (2d, 7d, 15d, and 30d, respectively) after ablation are shown in the upper row. Loading control with β-actin immunoreactivity is seen in the lower row. **B:** Graph showing the percentage changes of CB_1 expression after 2, 7, 15, and 30 days postablation in relation to the control optic tecta for each time point. Expression of CB_1 exhibited a statistically significant increase after 7 days ($\star P < 0.05$) and 15 days ($\star\star P < 0.01$).

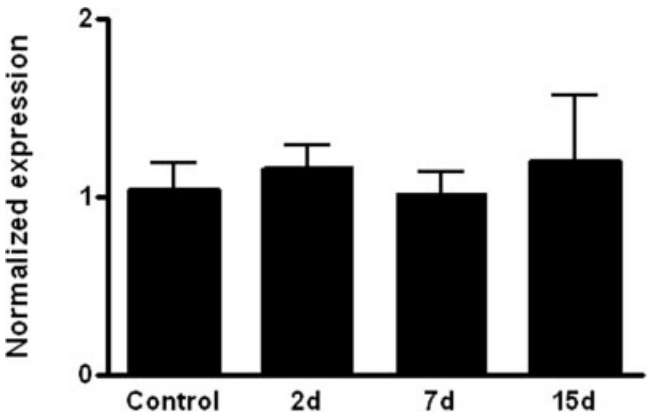

Fig. 5. Expression of CB_1 mRNA in the optic tectum evaluated by real-time PCR. The graph depicts the normalized CB_1 mRNA expression after 2, 7, and 15 days postablation in relation to the control optic tecta for each time point.

organization (Fig. 6). The MAP-2 increase was more noticeable after 7 days postlesion.

DISCUSSION

The data from the present study suggest that tectal deafferentation of its major afferent source induces an up-regulation of CB_1 that appears not to be dependent on increased mRNA levels. Although there is no evidence that the protein increase involves functional receptors, the CB_1 increase could be the result of reduced degradation or of mobilization of receptors from a precursor cytoplasmic pool, which is not recognized by the anti-CB_1 antibody under control conditions. The CB_1 up-regulation could be related either to cell death resulting from deafferentation and/or to tectal plasticity in response to retinal removal. Our data with TUNEL and Fluoro-Jade B suggest, however, that there is no cell death in the tectum in the present model. This appears to favor the hypothesis that the CB_1 up-regulation may be more related to plasticity processes that occur in the deafferented tectum.

The present data, besides adding information on the functional organization of the cannabinoid system, also generated information about the localization of the CB_1 receptors in the retinotectal system. These receptors are expressed only in the neuropil of superficial tectal layers (Leonelli et al., 2005) and, as such, they could constitute presynaptic receptors on retinal ganglion cell terminals. Indeed, retinal ganglion cells of the chick retina and other species do express CB_1 receptors (Straiker et al., 1999a,b), which could be exported to their central terminals. Alternatively, those receptors in the tectum could participate in plasticity events after the retinal removal as postsynaptic receptors in different tectal layers. Several studies have shown the presynaptic localization of cannabinoid receptors on GABA-ergic and glutamatergic neurons, suggesting a participation in plasticity processes (Freund et al., 2003; Piomelli, 2003). Although most of these studies indicate a presynaptic localization of cannabinoid receptors, the increase of the CB_1 expression in tectal layers of the chick brain after retinal ablations observed in the present study is suggestive of a postsynaptic location, maybe as part of the internal tectum circuitry. This hypothesis is corroborated by the fact that, even after longer survival times of the ablation (15 and 30 days), CB_1 expression still seems to be increased. Besides the general idea of a presynaptic location of cannabinoid receptors, other studies support the hypothesis of an intrinsic location of those receptors, because their authors did not find any changes of receptor binding or immunoreactivity in several hypothalamic and vestibular regions, respectively, after deafferentation (Romero et al., 1998; Ashton et al., 2004). However, we cannot exclude the possibility that CB_1 receptors are presynaptic on axon terminals from other tectal afferent sources. Likewise, the possibility that the retinal endocannabinoid system (Porcella et al., 1998; Bisogno et al., 1999; Yazulla et al., 1999) influences the tectal function remains to be investigated.

In a recent study from our laboratory, we showed a similar up-regulation effect for another chemically defined system, namely, an increase of the neuronal isoform of nitric oxide synthase in the tectum after retinal removal (Torrão and Britto, 2004). These data suggest

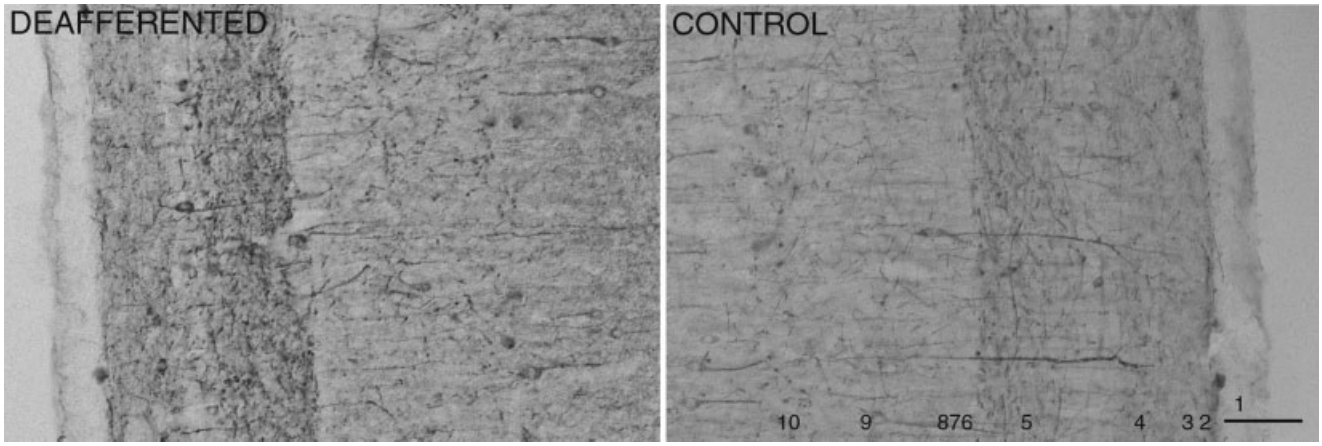

Fig. 6. Immunohistochemical staining for MAP-2 in the chick optic tectum 7 days after unilateral retinal ablation. Staining in the deafferented side is markedly increased. Numbers refer to tectal layers, according to Cajal's scheme. Scale bar = 200 μm.

that, similar to the nitrinergic system, the endocannabinoid system in the tectum is normally under negative regulation by retinal activity, and its up-regulation after retinal ablation may be involved in plasticity processes.

Many studies suggest the participation of the endocannabinoid system in neuroprotection and neurodegeneration processes by the use of different models of neuronal injuries both in vitro and in vivo (Hansen et al., 2002; Parmentier-Batteur et al., 2002; van der Stelt et al., 2002), and at least part of this protection seems to occur by the activation of the CB_1-type of cannabinoid receptors (Panikashvili et al., 2001, 2005; Parmentier-Batteur et al., 2002). For example, Panikashvili and collaborators (2001) have shown an increase in the levels of the endocannabinoid 2-arachidonoyl-glycerol (2-AG) after closed head injury in mice, and the beneficial effect of 2-AG was dose dependently attenuated by SR-141761A, an antagonist of the CB_1 cannabinoid receptor. In another study, it was suggested that CB_1 receptors have a role in neuroprotective processes, insofar as CB_1 receptor knockout mice showed an increased mortality after permanent focal cerebral ischemia (Parmentier-Batteur et al., 2002). However, the neuroprotective effects of the endocannabinoids are still under debate, because studies on cell cultures were not able to confirm that protective effect (Nilsson et al., 2003). Our results may corroborate the idea of a possible neuroprotective role of the endocannabinoid system and that part of this protection mechanism seems to be mediated by CB_1 receptor, insofar as this receptor was increased in the optic tectum after deafferentation. Panikashvili and collaborators (2001) showed a transient increase of the 2-AG levels after closed head injury in mice. They observed that the level of 2-AG was increased after the first hours, peaked about 4 hours after the lesion, and was still increased 24 hr after the lesion. Our data are in agreement with a transient protection hypothesis for the endocannabinoid system; we observed a peak of CB_1 expression after 7 days of survival time in visual structures. Several studies indeed demonstrate increased levels of endocannabinoids and up-regulation of CB_1 receptors in brain injury models in rats and mice (Romero et al., 2000; Hansen et al., 2001; Panikashvili et al., 2001; van der Stelt et al., 2001; Unzicker et al., 2005). For example, Hansen and collaborators (2001), using rats with mild neurodegenerative processes induced by changes of glutamatergic neurotransmission and of endocannabinoid homeostasis, found higher levels of anandamide, but not of 2-AG, and increased binding and mRNA of CB_1 receptors. Interestingly, Romero and collaborators (2000) found a significant increase of CB_1 mRNA levels, but not receptor binding, in basal ganglia of rats subjected to lesion of nigrostriatal dopaminergic neurons. Our results, on the other hand, revealed changes of CB_1 protein levels but not of the CB_1 mRNA after retinal ablation, suggesting a posttranscriptional mechanism of receptor mobilization. These distinct results found by different groups could be interpreted to mean that the regulation of the endocannabinoid system is very complex and/or depends on the model and brain area tested.

Although several recent studies support the neuroprotection effects of the endocannabinoid system in distinct neurotoxicity models, the exact mechanims involved in this process are still unknown (Marsicano et al., 2002). It is interesting to mention that Khaspekov and coleagues (2004) suggested a participation of neurotrophic factors in neuroprotection events triggered by the activation of CB_1 receptors.

The endocannabinoid system has also been pointed out as an important system in plasticity processes of the central nervous system. Karanian and collaborators (2005) describe the participation of CB_1 receptor in the generation of important signals for the maintenance of synapses in the hippocampus of rats. Then, the increase of CB_1 expression observed here could represent a participation of endocannabinoid system in the maintenance of synaptic integrity. Another study suggests a participation of CB_1 receptor in the control of the vestibular sys-

tem plasticity after unilateral deafferentation (Ashton et al., 2004). Unlike our present results, the latter authors suggest plasticity-related effects of CB_1 that do not require changes of protein expression. In addition, Tagliaferro and collaborators (2006) demonstrate changes in cytoskeletal and synaptic density elements after chronic treatment with a CB_1 receptor agonist. Taken together with the present data, this bulk of information contributes to link the endocannabinoid system to plasticity processes. In the present study, the increase of CB_1 receptor expression could thus be involved in a plastic remodeling of the visual system after retinal removal. It is very tempting to speculate that the increase of MAP-2 staining in much the same layers that express CB_1, and with a similar time course, could be dependent of an effect of these receptors on the dendritic reorganization that ensues after retinal removal. This hypothesis may be the subject of additional, combined pharmacological/morhological experiments.

In summary, our results suggest that CB_1 receptors in the optic tectum of the chick brain are negatively regulated by the retinal innervation. The increase of expression observed after retinal removal could be indicative of a participation of the endocannabinoid system in plasticity processes triggered by deafferentation.

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